translocation from DNA unwinding. Furthermore, this pin is structurally supported by interactions with a "tower" domain that locks it rigidly in place, creating an arch through which the ssDNA passes onto the core motor. Remarkably, simply destabilizing the pin by mutation of its interface with the tower domain caused substantial defects in DNA unwinding.

Unwinding pins have been observed in many other helicases (Figure 1), but their importance for DNA unwinding may vary, even in closely-related systems. The RecQ helicases provide an interesting example, as the size and apparent importance of the pin varies between family members. In human RecQL1, a conserved tyrosine residue at the tip of the pin is critical for coupling ATP hydrolysis to unwinding, whereas *E. coli* RecQ is rather tolerant of similar mutations (Pike et al., 2009). The pin can also adopt architectures other than the common beta-hairpin. In the SF2B helicase XPD, a duplex separation wedge is thought to be formed by an iron-sulfur domain associated with the 1A core domain and disruption of the cluster by mutagenesis uncouples DNA unwinding from ATP hydrolysis (Pugh et al., 2008; Kuper et al., 2012). In the bipolar helicase RecBCD, a putative pin is not found in either of the RecB or RecD helicase subunits, but is instead contributed by a short loop in the RecC polypeptide, which stacks a methionine dyad directly against the terminal base pair of the duplex (Singleton et al., 2004). Curiously, a secondary pin element is present in RecD, despite the fact that DNA arrives at this motor as pre-formed ssDNA. Finally, pin and wedge domains are not only a feature of DNA unwinding enzymes but also of motors that remodel branched DNA structures such as RecG and RuvAB. While the pin performs a similar role, in that it re-directs the duplex strands onto separate paths, the ssDNA immediately anneals with a new partner strand to promote Holliday junction or replication fork remodeling.

REFERENCES

Byrd, A.K., Matlock, D.L., Bagchi, D., Aarattuthodiyil, S., Harrison, D., Croquette, V., and Raney, K.D. (2012). J. Mol. Biol. *420*, 141–154.

He, X., Byrd, A.K., Yun, M.-K., Pemble, C.W., IV, Harrison, D., Yeruva, L., Dahl, C., Kreuzer, K.N., Raney, K.D., and White, S.W. (2012). Structure *20*, this issue, 1189–1200.

Kuper, J., Wolski, S.C., Michels, G., and Kisker, C. (2012). EMBO J. *31*, 494–502.

Pike, A.C., Shrestha, B., Popuri, V., Burgess-Brown, N., Muzzolini, L., Costantini, S., Vindigni, A., and Gileadi, O. (2009). Proc. Natl. Acad. Sci. USA *106*, 1039–1044.

Pugh, R.A., Honda, M., Leesley, H., Thomas, A., Lin, Y., Nilges, M.J., Cann, I.K., and Spies, M. (2008). J. Biol. Chem. *283*, 1732–1743.

Saikrishnan, K., Powell, B., Cook, N.J., Webb, M.R., and Wigley, D.B. (2009). Cell *137*, 849–859.

Singleton, M.R., Dillingham, M.S., Gaudier, M., Kowalczykowski, S.C., and Wigley, D.B. (2004). Nature *432*, 187–193.

Velankar, S.S., Soultanas, P., Dillingham, M.S., Subramanya, H.S., and Wigley, D.B. (1999). Cell *97*, 75–84.

Regulated Proteolysis as a Force to Control the Cell Cycle

Jodi L. Camberg[1,*] and Sue Wickner[1,*]
[1]Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 USA
*Correspondence: cambergj@mail.nih.gov (J.L.C.), wickners@mail.nih.gov (S.W.)
http://dx.doi.org/10.1016/j.str.2012.06.004

In this issue of *Structure*, Rood and colleagues report that substrate architecture is a key factor in promoting the complete and processive degradation of the *Caulobacter* cell cycle regulator PdeA by the protease ClpXP. This investigation highlights the important role that the adaptor protein CpdR serves in regulating presentation of PdeA to ClpXP.

The degradation of cell cycle regulators during the cell division cycle of *Caulobacter crescentus* is critical for controlling the timing of events such as DNA replication and cell division (Curtis and Brun, 2010). A driving force for the programmed transition from G1 to S-phase is ClpXP, a two-component ATP-dependent protease. Through degradation of the cell cycle regulator, CtrA, and the phosphodiesterase PdeA, ClpXP orchestrates entry into S-phase (Chien et al., 2007; Jenal and Fuchs, 1998).

In this issue of *Structure*, Rood et al. (2012) describe structural organization and examine regulated degradation of PdeA. PdeA antagonizes the activity of DgcB, a diguanylate cyclase (Abel et al., 2011). When PdeA is degraded by ClpXP, DgcB is left unchecked, creating a situation that causes an upshift in cyclic-di-GMP and leads to CtrA degradation. PdeA degradation by ClpXP requires an adaptor protein, CpdR, which is also a response regulator. CpdR functions in the traditional role of a substrate-specific adaptor protein, facilitating the degradation of PdeA.

ClpXP is a member of the protein quality control machinery with structural organization and a mechanism of degradation similar to the eukaryotic 26S proteasome (Baker and Sauer, 2012; Ortega

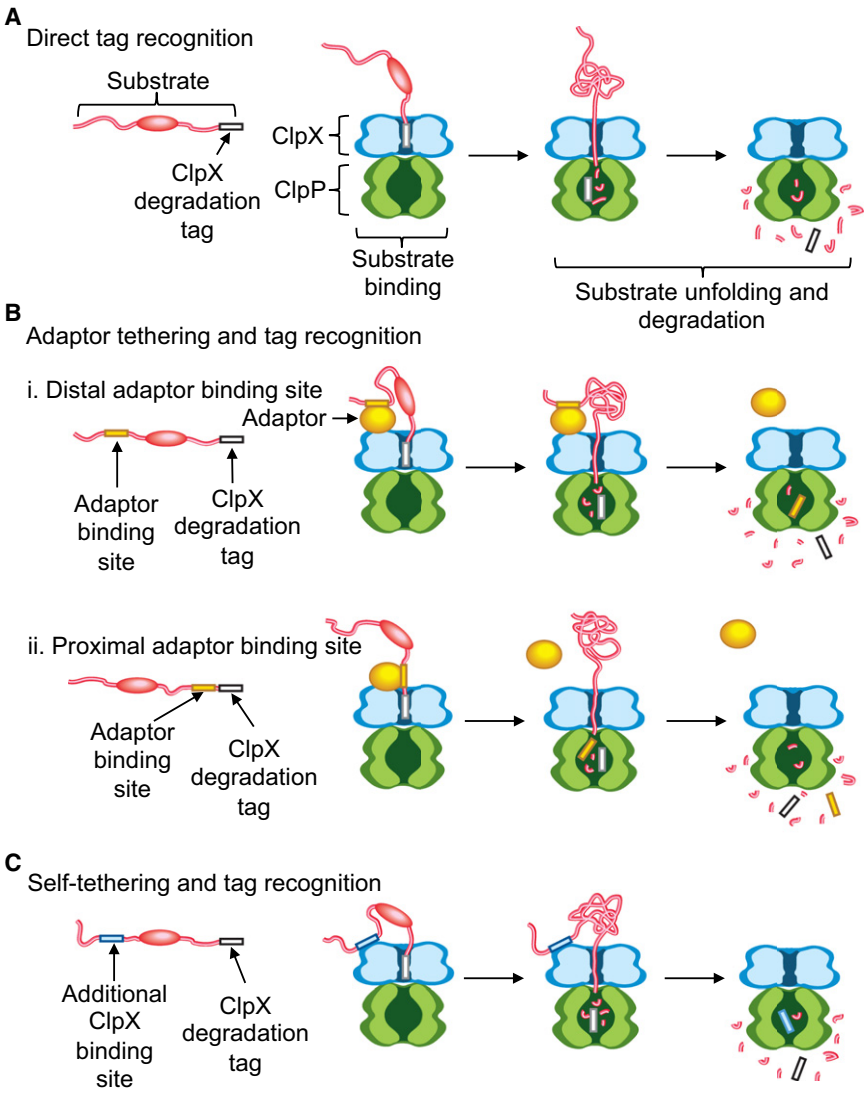

Figure 1. Substrate Architecture Drives Degradation by ClpXP
(A) Substrate recognition by ClpX directly through a degradation tag. By this mechanism, a high affinity recognition signal at the C- or N terminus of the substrate interacts with ClpX and initiates protein unfolding and degradation.
(B) Substrate recognition through an adaptor protein. Recognition is mediated by a ternary interaction between the substrate, an adaptor protein, and ClpX that facilitates the delivery of the substrate to ClpX. The adaptor site may be located either distal, as in the case of PdeA (i), or proximal (ii) to the ClpX binding site.
(C) Substrate recognition through self-tethering. Here, the substrate has a second ClpX recognition site that interacts with ClpX and stabilizes the interaction between ClpX and a relatively low affinity ClpX binding site.

et al., 2002; Sauer and Baker, 2011). ClpX is a AAA+ domain containing protein that has a high degree of similarity to other ATP-dependent unfolding proteins, such as ClpA and ClpB/Hsp104. ClpX assembles into a hexameric ring with a central pore (Glynn et al., 2009). This remarkable unfoldase harnesses the power of ATP hydrolysis, thus coupling energy release to conformational changes in ClpX that force the substrate to unfold and transit through the central pore of the hexamer. The unfolded polypeptide advances through the channel by engaging conserved substrate-binding loops in the ClpX pore and is met at the exit by ClpP. ClpP, a serine protease, is comprised of two stacked hexameric rings of identical subunits forming an enclosed chamber with the proteolytic active sites lining the interior of the chamber. ClpX is situated over the entrance to the ClpP chamber, tightly regulating access to ClpP and thus degradation.

In the simplest scenario, proteins fated for proteolysis by ClpXP are recognized by ClpX directly (Figure 1A). A short peptide extension of about a dozen amino acids at either the N- or C terminus of the substrate is recognized and bound by ClpX. The tag sequence interacts with residues in the ClpX pore, thereby positioning the substrate to be threaded through ClpX and into the ClpP cavity for degradation. The SsrA-peptide is an example of a tag that is directly recognized by ClpX. It is an 11 amino acid peptide added cotranslationally to the C terminus of proteins stalled on ribosomes that target the damaged proteins for degradation by ClpXP (Gottesman et al., 1998). Several specific ClpXP substrates are similarly recognized directly by a terminal tag.

The use of adaptor proteins to promote recognition of substrates by ClpX provides an additional level of regulation that the cell can exploit to control degradation under specific conditions (Ades, 2004) (Figure 1B). Adaptor proteins interact with both ClpX and the substrate and function to promote the productive engagement of the substrate by ClpX. The adaptor binding site can be present at a location distal to the ClpX degradation tag, or degron (Figure 1Bi), or in a position that is proximal to the ClpX degradation tag (Figure 1Bii).

Determination of the architecture of PdeA described by Rood et al. (2012) revealed that PdeA contains an adaptor binding site that is very distant from the ClpX degradation tag (an example of a distal adaptor binding site is depicted in Figure 1Bi). The authors show that PdeA consists of an N-terminal PAS-like (Per-Arnt-Sim) domain followed by an altered GGDEF domain (GEDEF) that binds GTP and activates cyclic-di-GMP phosphodiesterase in the neighboring C-terminal EAL domain. Dimerization of PdeA is mediated by an N-terminal α-helix; although its removal prevents PdeA dimerization, it does not prevent ClpXP degradation. The adaptor binding site is in the N-terminal PAS domain, whereas degradation initiates from a C-terminal ClpX degradation tag. This architectural arrangement with the

adaptor binding site at one end and the ClpX degradation tag at the opposite end effectively stabilizes the interaction between ClpX and PdeA. The authors further suggest that this interaction between the N-terminal domain of PdeA and CpdR enhances the processivity of degradation, supported by the result that the presence of the adaptor CpdR increases the V_{max} of the degradation reaction by 30-fold. Therefore, the interaction between the adaptor and the substrate is the driving force for the degradation originating at a weak degradation tag.

With some substrates, the adaptor binding site is proximal with respect to the position of the ClpX degradation tag (Figure 1Bii), as in the case of the SsrA-tag. As mentioned above, the SsrA-tag supports direct recognition by ClpX. However, the N-terminal half of the tag also supports an interaction with the adaptor protein, SspB (Sauer and Baker, 2011). SspB binds to SsrA-tagged proteins and the N-domain of ClpX, enhancing recognition and thus facilitating unfolding and degradation of SsrA-tagged proteins by ClpXP.

Other substrates utilize a key feature of adaptor-mediated recognition, the ability to tether the substrate to ClpX, but without an external adaptor protein. In this example of self-tethering, the substrate incorporates regions located at positions other than a terminus, which also interact with ClpX. These additional contacts promote engagement of the substrate by ClpX, leading to substrate unfolding (Figure 1C). One example is the phage Mu transposase, MuA, which contains a ClpX degradation tag at its C terminus as well as additional residues that make extended contacts with ClpX to stabilize the association (Abdelhakim et al., 2008).

Regulated proteolysis is essential for development in many organisms. The work presented by Rood et al. (2012) helps clarify the contribution of proteolysis to the *Caulobacter* cell cycle. The incorporation of regulated proteolysis into an already complex regulatory network, such as the transition from G1 to S-phase in *Caulobacter* enables the cell to precisely control the functional activities of cellular components. Rood et al. (2012) present the structural characterization of an adaptor binding domain, thus providing mechanistic insight into how substrates are selected and recognized by cellular proteases.

ACKNOWLEDGMENTS

Work in the authors' lab was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

REFERENCES

Abdelhakim, A.H., Oakes, E.C., Sauer, R.T., and Baker, T.A. (2008). Mol. Cell *30*, 39–50.

Abel, S., Chien, P., Wassmann, P., Schirmer, T., Kaever, V., Laub, M.T., Baker, T.A., and Jenal, U. (2011). Mol. Cell *43*, 550–560.

Ades, S.E. (2004). Curr. Biol. *14*, R924–R926.

Baker, T.A., and Sauer, R.T. (2012). Biochim. Biophys. Acta *1823*, 15–28.

Chien, P., Perchuk, B.S., Laub, M.T., Sauer, R.T., and Baker, T.A. (2007). Proc. Natl. Acad. Sci. USA *104*, 6590–6595.

Curtis, P.D., and Brun, Y.V. (2010). Microbiol. Mol. Biol. Rev. *74*, 13–41.

Glynn, S.E., Martin, A., Nager, A.R., Baker, T.A., and Sauer, R.T. (2009). Cell *139*, 744–756.

Gottesman, S., Roche, E., Zhou, Y., and Sauer, R.T. (1998). Genes Dev. *12*, 1338–1347.

Jenal, U., and Fuchs, T. (1998). EMBO J. *17*, 5658–5669.

Ortega, J., Lee, H.S., Maurizi, M.R., and Steven, A.C. (2002). EMBO J. *21*, 4938–4949.

Rood, K.L., Clark, N.E., Stoddard, P.R., Garman, S.C., and Chien, P. (2012). Structure *20*, this issue, 1223–1232.

Sauer, R.T., and Baker, T.A. (2011). Annu. Rev. Biochem. *80*, 587–612.

Spotting the Mistakes, One Molecule at a Time

Audrey Quessada-Vial[1] and Antoine M. van Oijen[1,*]
[1]Zernike Institute for Advanced Materials, Groningen University, Nijenborgh 4, 9747 AG Groningen, The Netherlands
*Correspondence: a.m.van.oijen@rug.nl
http://dx.doi.org/10.1016/j.str.2012.06.007

In this issue of *Structure*, Cho and colleagues provide intriguing insight into the first steps of the DNA mismatch repair process. By using single-molecule techniques, they show that the protein MutS undergoes two different types of diffusion on error-containing DNA in an ATP-dependent way.

The DNA mismatch repair (MMR) pathway is a highly conserved process that helps to maintain the integrity of the genome. One of its key functions is the recognition and repair of mismatches that are introduced during DNA replication and that are not repaired by the proof-reading activity of the DNA polymerase. In this issue of *Structure*, Cho et al. (2012) describe an elegant single-molecule approach to directly observe the very first step of the bacterial MMR pathway, the detection of the error by a protein called MutS.

Bacterial MutS is composed of seven domains, including an ATPase, a clamp, and a mismatch-binding domain and can form homodimers and tetramers.